NZY Viral RNA Isolation kit

Catalogue number: MB40701, 50 columns

Description

NZY Viral RNA Isolation kit is designed for the rapid and simple purification of viral RNA of the highest integrity from a variety of sources, in particular serum, plasma, saliva, nasal samples, blood, tissue, organs and environmental samples. Although high yields are to be expected from the implementation of this protocol, for sensitive applications requiring the highest performance in terms of RNA recovery please select NZY Total RNA Isolation kit (MB13402). This method uses a denaturing lysis buffer containing guanidine thiocyanate, which also inactivates cellular RNases, to ensure the recovery of intact RNA molecules. Ethanol is subsequently added to provide selective binding of nucleic acids into the silica membrane column and impurities are efficiently washed away. High-quality viral RNA is then eluted in RNase-free water. Viral RNA is ready to use for detection using different amplification strategies such as Reverse Transcription (RT)-PCR or RT-qPCR. NZY Viral RNA Isolation kit is optimized to isolate RNA with high purity/integrity displaying an $A_{260/280}$ ratio between 1.9 and 2.1. Traces of DNA may be co-purified with the viral RNA. Please treat the RNA sample with DNAse to ensure complete removal of DNA trace contaminations.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (20-25 °C) and are stable until the expiry date. Before use, add 40 mL of molecular biology grade absolute ethanol (96-100 %) to

the NVW bottle. Buffers NVL and NV contain guanidine salts. Wear gloves and goggles when using this kit.

System Components

Component	Volume (50 preps)
Buffer NVL	20 mL
Buffer NV	12.5 mL
Buffer NVW (concentrate)	10 mL
RNase-free Water	7 mL
NZYSpin Virus Columns	50
Collection tubes (2 mL)	50

Guidelines for using NZY Viral RNA Isolation kit

- RNA preparation using NZY Viral RNA Isolation kit can be performed at room temperature. Particular care should be taken with isolated RNA, since it is highly sensitive to degradation by trace contaminations of RNases. Thus, develop efficient laboratory practices during the whole purification process to avoid contaminating the sample with RNases. **Wear gloves at all times during RNA preparation and change gloves frequently**. Use RNase-free plasticware and reagents. To ensure RNA stability, store pure RNA at -20 °C for short-term or at -70 °C for long-term.
- Before start, ensure that absolute ethanol (40 mL) was added to Buffer NVW.

Protocol for purification of viral RNA

I. Sample preparation

Swab samples: Place the oral, nasal or pharyngeal swab directly into 500 µL of sterile saline solution (e.g. PBS buffer) and take the supernatant for RNA isolation. Alternatively introduce the swab in transport medium for preservation. Proceed with step II.

Saliva samples: Mix 285 µL saliva with 15 µL of a 20 mg/mL Proteinase K solution (not provided). Vortex vigorously and incubate at room temperature for 2-3 minutes. Proceed with step II. 2.

Tissue samples: Cut up to 100 mg tissue sample into small pieces and place them in a RNase-free microcentrifuge tube. Proceed with step II. 2.

II. Preparation of viral RNA

1. We recommend to use 100-200 µL of the processed samples for each preparation. If using larger volumes, please scale up the volume of lysis buffer and ethanol subsquently added to the sample before loading; the amounts of Buffers NV and NVW used in the wash steps of the silica column do not need to be increased.

2. Add 350 µL of Buffer NVL to the processed sample. Vortex vigorously and incubate for 10 min at room temperature (15-25 °C).

Notes:

- *The lysate may be passed through a needle fitted to a syringe to reduce viscosity;*
- *Difficult-to-lyse samples can be incubated for 15-20 minutes in a water bath at 65 °C.*

3. Add 350 µL of absolute ethanol (96-100 %) and mix immediately by pipetting up and down or by vortexing. **Do not centrifuge the sample at this stage**. (*Note*: To ensure efficient binding, it is crucial that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.)
4. Carefully apply 700 µL of the lysed sample into an NZYSpin Virus column placed in a 2 mL collection tube. Centrifuge for 1 min at 8,000 x*g*. Discard the flow-through and place the column back into the collection tube.

Note: *The use of a new 2 mL collection tube is recommended if infectious material has to be prepared.*

5. If the lysed sample volume was greater than 700 µL, repeat step 4 until all of the lysate has been loaded onto the spin column.
6. Add 200 µL of Buffer NV and centrifuge for 1 min at 8,000 x*g*. Discard the flow-through and place the column back into the collection tube.
7. Add 600 µL of Buffer NVW and centrifuge for 1 min at 8,000 x*g*. Discard the flow-through and place the column back into the collection tube.

Note: *It is not necessary to increase the volume of Buffer NVW even if the original sample volume was increased.*

8. Repeat wash with 300 µL of Buffer NVW and centrifuge for 1 min at 8,000 x*g*. Discard the flow-through and place the column back into the collection tube.
9. Centrifuge for 2 min at 8,000 x*g* to dry the column membrane. Discard the flow-through.
10. Place the NZYSpin Virus column in a clean 1.5 mL RNase-free microcentrifuge tube. Add 50-100 µL RNase-free water directly to the column membrane and incubate at room temperature for 2-5 mins.

Note: *Pre-warming water at 65 to 70 °C may increase elution efficiency.*

11. Centrifuge for 1 min at 8,000 x*g* to elute RNA from the column.
12. Store the RNA at -20 °C for short-term or at -70 °C for long-term.

Quality control assay

Functional assay

All components of NZY Viral RNA Isolation Kit are tested following the isolation protocol described above.

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For life science research only. Not for use in diagnostic procedures.